(open circles) and the fitted function according to a function of formula I; X-axis: time [min]; Y-axis: conductivity [mS/cm].

FIG. **3** Absolute difference between the experimental data for an exemplary inert change of the conductivity for a re-usable chromatography column packing with no reduced separation efficacy/packing quality and the fitted function according to formula II; X-axis: time; Y-axis: difference.

FIG. **4** Experimental data for an exemplary inert change of the conductivity for a re-usable chromatography column packing with reduced separation efficacy/packing quality (open circles); X-axis: time [min]; Y-axis: conductivity [mS/cm].

FIG. **5** Experimental data for an exemplary inert change of the conductivity for a re-usable chromatography column packing with reduced separation efficacy/packing quality (open circles) and the fitted function according to a function of formula I; X-axis: time [min]; Y-axis: conductivity [mS/cm].

FIG. **6** Absolute difference between the experimental data for an exemplary inert change of the conductivity for a re-usable chromatography column packing with reduced separation efficacy/packing quality and the fitted function according to a function of formula II; X-axis: time [min]; Y-axis: difference [mS/cm].

FIG. **7** Fitting of conductivity data used in FIG. **1** to formula III employing Gaussian distribution without the contribution of equipment characteristics:

$$yIII = \frac{1}{2}P1 \cdot \left(1 + erf\left(\frac{x - x_c}{w \cdot \sqrt{2}}\right)\right) + A0 \qquad \text{formula III}$$

Upper part: Data and fitted curve, lower part: difference curve of data from fitted curve.

X-axis: time [min]; Y-axis: conductivity [mS/cm].

FIG. **8** Monitoring of column integrity with a method as reported herein over 250 chromatographic cycles using formula I (depicted as EMG) and formula III (depicted as Gauss). Integrity parameters shown are x_c (retention time), w (peak broadening) and t_0 (equipment characteristics). In the figure for t_0 column repacks that became necessary due to decreased packing quality are indicated by vertical bars in light grey.

FIG. **9** Monitoring of column integrity with a method as reported herein over 250 chromatographic cycles using formula I (depicted as EMG) and formula III (depicted as Gauss). Quality of fit parameters shown are X^2 (chi-square), R^2 (R-factor) and residuals difference normalized to maximum conductivity as described for formula II.

EXAMPLE 1

Fermentation and Purification of Erythropoietin

Erythropoietin can be produced and purified e.g. according to WO 01/87329.

The purification comprises some chromatography steps. One of these is a Blue Sepharose chromatography. Blue Sepharose consists of Sepharose beads to the surface of which the Cibacron blue dye is covalently bound. Since erythropoietin binds more strongly to Blue Sepharose than most non-proteinaceous contaminants, some proteinaceous impurities and PVA, erythropoietin can be enriched in this step. The elution of the Blue Sepharose column is performed by increasing the salt concentration as well as the pH. The column is filled with Blue Sepharose, regenerated with NaOH and equilibrated with equilibration buffer (sodium/calcium chloride and sodium acetate). The acidified and filtered fermenter supernatant is loaded. After completion of the loading, the column is washed first with a buffer similar to the equilibration buffer containing a higher sodium chloride concentration and consecutively with a TRIS-base buffer. The product is eluted with a TRIS-base buffer and collected in a single fraction in accordance with the master elution profile.

During the equilibration, separation, and regeneration step of the chromatography cycle the conductivity of the mobile phase at the outlet of the column is determined and recorded with a standard conductivity measuring device.

Fermentation and Purification of an Anti-HER2 Antibody

An anti-HER2 antibody can be produced and purified e.g. according to U.S. Pat. No. 5,821,337 or U.S. Pat. No. 5,677,171.

Antibody in harvested cell culture fluid samples can be captured and purified using a specific affinity chromatography resin. Protein A resin (Millipore, Prosep-vA High Capacity) was selected as the affinity resin for antibody purification. The resin was packed in a column.

The resin was exposed to buffers and harvested cell culture fluid (HCCF) at a linear flow rate between 260-560 cm/hr. The resin was equilibrated with 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1.

For each purification, the resin was loaded between 5-15 mg antibody per mL of resin. After loading, the resin was washed with 25 mM Tris, 25 mM NaCl, 5 mM EDTA, 0.5 M TMAC, pH 5, and then the antibody was eluted using 0.1 M citric acid, pH 2.8. Elution pooling was based on UV absorbance at 280 nm measured inline after the column. The purified elution pools were pH-adjusted using 1 M TRIS buffer to pH 5-6. After regeneration of the resin with 0.1 M guanidinium hydrochloride, the same or similar packed resins were used for subsequent purification of other HCCF solutions.

The antibody concentration in the purified protein A pool was measured using UV spectrometry at 280 nm. The purified protein A elution pools were analyzed by size exclusion chromatography to quantitate the percentage of intact antibody at 150 kDa molecular weight.

EXAMPLE 2

Change in Column Properties

The column can be monitored over the process continuously using the method as reported herein. Subtle changes become detectable independently of changes of other process parameters. In FIGS. **8** and **9** changes in the column packing are shown over the complete lifetime of the Prosep A columns used in the purification of an anti-HER2 antibody. In those cases, where repacking became necessary, there is a clear indication of changes in the parameters derived from packed bed quality (see. FIG. **8**).

The invention claimed is:

1. A method for determining whether a re-useable chromatography column packing, which is used at least for the second time in a purification step of a purification of a polypeptide, has reduced separation efficacy in said purification step of said purification of said polypeptide, characterized in that said method comprises the following steps:

a) identifying and determining the experimental data of an inert change of at least one physicochemical parameter of a mobile phase passing through said re-useable chromatography column packing of the at least second use,